REMARKS

The present application relates to a method for Agrobacterium-mediated transformation of explants from fibrous roots of young cotton seedlings. The fibrous roots are obtained from seedlings that are cultured in a medium which includes multi-effect triazole (MET), a chemical agent used in agriculture to promote root growth. Applicants of the present application have unexpectedly discovered that MET reduces browning of the cotton seedling roots and thereby increases the rate of callus initiation such that it is possible to use fibrous roots of young seedlings as an explant tissue for cotton transformation.

Claims 1-30 are pending in the application. In this response Applicants correct several typographical errors discovered in the specification. They also submit new Claims 31 to 36 and cancel Claims 1-3, 9, 12, 27 and 29. Claims 4-8, 10-11, 13-16, 18, 20-26, 28 and 30 are amended to correct dependency and as to correct several typographical errors.

Objection Under 37 CFR § 175(c)

Claims 26 and 27 are objected to as being in improper form, as a multiple dependent claim should depend upon other claims in the alternative only. In this response claim 26 is amended to correct the multiple dependency. Claim 27 is canceled. Applicants respectfully ask that the objection under 37 CFR § 175(c) be withdrawn.

Rejection Under 35 U.S.C. §112, Second Paragraph

Claims 1-30 are rejected under 35 U.S.C. § 112, second for the reasons discussed below. Applicants respectfully ask that this rejection be withdrawn in view of the individual corrections or clarifications made to address the Examiner's objections.

The term "fibrous root explants" in step (a) of Claim 1 is asserted to be unclear. When read in the context of the specification the term "fibrous" refers to the root of a cotton seedling, and not to the explant. See for example, at least page 6 at lines 13-14 and page 12, lines 7 to 12. New independent claim 31 refers to explants from fibrous roots of cotton seedlings, which Applicants believe clarifies the term "fibrous root explants."

The Examiner has noted that the term "root callus" in step (c) lacks antecedent basis. New Claim 31 is drafted to make clear that it is the callus of step (b) that is referred to in step (c).

The Examiner has asserted that the term "gene" is unclear as the term could imply a DNA sequence that could exist in nature. With this amendment Applicant submits new claim 31 which recites "a DNA encoding a chimeric gene of interest." Support for this term is found beginning at page 8, line 20 through page 9, line 32.

Applicants respectfully disagree that the term "about" used in claims 2-30 is unclear. The use of terms such as "about" to indicate that the concentration of the components of the various medium of the present application may vary over a range as in claims 6, 8, 11, 15, 22, 24 and 28 containing such concentrations will be readily understood by one of skill in the relevant art. Applicants respectfully ask that the rejection of the claims containing the term "about" be withdrawn.

As evidenced by the Liang et al. reference cited by the Examiner, multi-effect triazole is a chemical readily known to those of skill in the applicable art. Thus, applicants respectfully ask that the Examiner withdraw her objection that the term "multi-effect triazole" is indefinite.

The terms "the selected callus culture" and "the selected somatic embryos" no longer appear in new claim 31 or in any claim which depends therefrom either directly or indirectly.

Claims 5 and 11 have been reworded to make the term "the additional presence" unnecessary.

Rejection Under 35 U.S.C. §112, Enablement

Claims 1-30 have been rejected under 35 U.S.C. § 112, first paragraph as failing to comply with the enablement requirement. Applicants respectfully traverse this rejection as it may be applied to the claims as presently amended and to new claims 31-37. The present claims are drawn to an improved method for obtaining transgenic cotton plants. The details of the specification of the present application provide sufficient direction both by way of a detailed written description and examples to allow those of the relevant art to practice the invention.

Applicant's claims are limited to a particular strain of Agrobacterium, *Agrobacterium tumefaciens*, albeit, the strain commonly used for plant transformation, including transformation of cotton. It is well within the skill of those workers in this art to select particular known vectors useful for transforming cotton plants with *Agrobacterium tumefaciens*.

Those of skill in the art of the production of transgenic plants are aware of conventional methods of plant breeding which involve crossing different plant varieties. Thus, a working example of a single species of cotton is sufficient to enable the present method. Once a chimeric gene of interest is inserted into a single variety using the methods of the present application, it can readily be moved to other varieties utilizing conventional methods of plant breeding. As explained in the present application at page 10, lines 14 to 21, Applicant's new method overcomes or minimizes several of the problems associated with previous work relating to cotton transformation through the use of fibrous root explants. Applicants respectfully assert that undue, trial and error experimentation would not be required to carry out the method of the present invention as presently claimed. They respectfully ask that the rejection under 35 U.S.C. § 112, first paragraph, as it may be applied to the present claims, be withdrawn.

Rejection under 35 U.S.C. §102

Claim 1 is rejected under 35 U.S.C. § 102(b) over PCT publication WO 97/12512, which corresponds to U.S. Patent No. 5,846,797 issued to Strickland. Applicants respectfully traverse this rejection as it may be applied to the present claims.

The Strickland publication relates to a method for regenerating cotton plants from explant tissue where the explant is not cultivated on cotton initiation medium having exogenous plant hormones. The application asserts, but does not show that the method is applicable to numerous kinds of cotton tissue, including root at page 11, lines 12 - 15 and in claim 11. The explants described in the examples of the Strickland application are from hypocotyls. There are no actual examples of transformation of callus derived from root explants and the regeneration of whole transgenic plants. Moreover, Strickland is silent on the use of multi-effect triazole and naphthalene acetic acid to obtain explants of the fibrous root tissues of cotton seedlings which are more resistant to browning and therefore have an increased initiation rate.

To anticipate a claim a reference must disclose each element of the claim. The claims of the present application are directed to the specific tissues--explants of fibrous roots of cotton seedlings--which the inventors exemplify in their application. The teachings of the Strickland reference fails to anticipate the claims of the present application as it does not disclose or even suggest the subject matter of those claims. Applicants respectfully ask that the rejection under 35 U.S.C. §102(b) over Strickland be withdrawn.

Rejections under 35 U.S.C. §103(a)

Claims 1-4 and 8-10 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Strickland, WO 97/12512, in view of Liang, et al. *Acta Agronomica Sinica*, 1997, vol. 23, no. 2, pages 220-225, and what is asserted to be applicant's

admitted prior art. Applicants respectfully ask that this rejection be with drawn as it may be applied to the present claims.

The claims of the present application are directed to a method of producing a transgenic cotton plant using explants of fibrous roots of seedlings. As discussed above, the Strickland publication does not describe or even suggest the method for producing a transgenic cotton plant of the present application. The Liang abstract merely describes the use of a multi-effect triazole in medium for *Triticum aestium* (i.e., wheat) anther culture to test its effects on culture responses. Nothing in Liang suggests that the use of a multi-effect triazole would produce explants of fibrous roots of cotton seedlings having an improved rate of callus formation.

Because the combination of Strickland and Liang does not disclose or even suggest the present invention, the combination of these references does not establish a *prima facia* case of obviousness. Nothing in Applicant's specification at pages 1-2 discloses or suggests the invention of the present claims. Therefore, Applicants respectfully ask that the rejection over Strickland, in view of Liang et al., be withdrawn as it may apply to the present claims.

Claims 1 and 14-30 are rejected over Strickland in view of Firoozabady et al., *Plant Molecular Biology* 10 (1987) pp. 105-116. It is acknowledged that Strickland does not teach use of multi-effect triazole or dimethylallyaminopurine. However, it is asserted that Firoozabady et al. teaches the use of medium identical to applicants' CB-2-1 medium, including naphthalene acetic acid, dimethylallylaminopurine, 2,4-D, $MgCl_2$, glucose, gellan gum and/or $NaNO_3$. Firoozabady does not disclose or even suggest the use of roots of seedlings as a source of explants, nor does it suggest the use of multi-effect triazole.

To establish a *prima facia* case of obviousness, the cited prior art must disclose or suggest each element of the claimed invention. The combination of Strickland and Firoozabady does not teach or even suggest the invention of the present claims. The disclosure of Strickland, which is directed to an improved method for generation of

embryogenic callus from a cotton hypocotyl explant, which is **not** cultivated on cotton initiation medium having exogenous plant hormones, teaches away from Firoozabady and from the present invention.

Firoozabady reports a method for transformation of cotton where cotyledon pieces, not callus derived from explants of root tissue, are incubated with *Agrobacterium*, followed by incubation in callus initiation medium.

Again, the combination of the teachings of Strickland and Firoozabady does not disclose or even suggest Applicant's method as presently claimed. Applicants respectfully ask that the rejection of claim 1 and 14-30 under 35 U.S.C. § 103(a), as it may be applied to the present claims, be withdrawn.

Claims 1-30 are rejected over Strickland in view of Firoozabady et al., further in view of Liang et al. (abstracts). Neither Strickland, Firoozabady or Liang taken alone or in any combination discloses or suggests the method for producing a transgenic plant of the present claims. These references, taken alone or in combination, do not suggest a method for making a transformed cotton plant using explants of fibrous root tissue of young cotton seedlings which are treated with multi-effect triazole to reduce browning of the root tissue and to increase the rate of callus formation. As discussed above Firoozabady treated cotyledon pieces with *Agrobacterium*, then induced callus formation in a plant hormone. Strickland teaches the regeneration of cotton plants from explant tissues that are not cultivated on medium having exogenous plant hormones. Liang et al. report studies of the application of multi-effect triazole in anther cultures of wheat. Nothing in these references disclose or even suggest the methods of the present claims. Applicants respectfully ask that the rejection of claims 1-30 over Strickland, in view of Firoozabady et al., and further in view of Liang et al. under 35 U.S.C. § 103(a) be withdrawn as it may apply to the present claims.

Applicants believe the present claims are in condition for allowance and respectfully request a timely notice to that effect. Should additional issues arise that can be effectively dealt with in a timely discussion with Applicant's representative, the

Examiner is respectfully asked to contact the undersigned Representative so that the case can be quickly passed to issue.

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Respectfully submitted,

By 

Barbara Webb Walker
Attorney for Applicants
Registration No. 35,400
ROTHWELL, FIGG, ERNST & MANBECK, P.C.
Suite 800, 1425 K Street, N.W.
Washington, D.C. 20005
Telephone: (202)783-6040